Attorney Docket No. 59150-8008.US00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:	
Ishikawa, *et al.*	EXAMINER: Brown, S.
SERIAL NO.: 09/555,704	ART UNIT: 1648
FILED: June 2, 2000	CONFIRMATION NO.: 9123
FOR: ENHANCED IMMUNOGEN FOR INACTIVATED VACCINE FOR INFECTION WITH JAPANESE ENCEPHALITIS VIRUSES AND PROCESS FOR PRODUCING SAME	

Declaration Under 37 C.F.R. §1.132
(MPEP §716.10)

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Commissioner for Patents
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Sir:

I, Asato Kojima, PhD, declare and affirm as follows:

1. I have been active in the field of virology research for over 15 years and have published extensively in this field (see Curriculum Vitae Attachment 1).

2. I have read the above-noted application, the Office action dated June 30, 2003, and the cited Huiying, *et al.* (Virologica Sinica, 13(3):208-213, 1998), Liau *et al.* (U.S. Patent No. 6,207,439) documents. I am also familiar with and have read the Chenghua *et al.* (Journal of Chinese Biological Products, 6(1):170-174, 1993) document referenced in Huiying *et al.*

The Invention

3. The inactivated virus particle of the present invention , achieves a neutralizing antibody titer of the anti-serum obtained by immunization with the virus particles that is about twice to about 10 times the neutralizing antibody titer of the anti-serum obtained by immunization with inactivated virus particles prepared from virus cultured in mouse brain, as evidenced by the data presented in Example 4 of the specification (page 24, beginning at line

22). The data shows that virus particles prepared by the claimed process can maintain higher immunogenicity or antigenicity, as compared with particles prepared by the prior art methods of purification followed by inactivation.

The inactivated virus particle is prepared by a process comprising a step of inactivation followed by a step of purification solely by physical means. Virus particles prepared by this process have an unaltered surface that preserves the correct steric conformation for presentation of the antigen to antibodies, leading to the high neutralizing antibody titer. This steric conformation can be seen in the electron micrograph of Fig. 1A of the specification. As seen, a virus particle prepared by the present process of inactivation followed by physical purification has a rough or fuzzy envelope layer surface (see page 8, lines 3-6; page 5, lines 27-30 of specification). Fig. 1B further shows an electron photomicrograph of a virus particle prepared by prior art, commercial methods where the virus is purified and then inactivated (page 5, lines 27-30; page 7, lines 1-4). As seen in Fig. 1B, the surface of these particles is smooth (page 8, lines 3-5).

Huiying *et al.*

4. Huiying *et al.* discloses a method for large-scale purification of inactivated JEV from Vero cells. Huiying *et al.* prepares the vaccine using the method of Chenghua, *et al.* The Chenghua *et al.* reference discloses that the virus is cultured in Vero cells, harvested and then inactivated with formalin.

Therefore, Huiying *et al.* discloses that zonal centrifugation is used to purify a concentrated vaccine which has already undergone inactivation, ultrafiltration, concentration, and protamine sulfate precipitation. The zonal centrifugation runtime and sucrose gradient concentration were optimized to obtain the most efficient virus separation.

The purpose of the protamine sulfate precipitation is to remove Vero cell DNA. The purpose of the zonal centrifugation is to remove non-viral protein.

Therefore, in the methodology of Huiying *et al.* a step of inactivation is followed by a chemical treatment step (protamine sulfate precipitation), before the step of zonal centrifugation.

Liau *et al.*

5. Liau *et al.* discloses a method for large-scale purification of living JEV from a JEV source. The viral preparation resulting from this method has low levels of non-viral protein and non-viral DNA and a high viral titre.

The examples disclosed in Liau *et al.* describe that JEV from mouse brain or cell culture is purified by microfiltration, ultrafiltration, and liquid chromotography and then subsequently inactivated. Liau *et al.* does not distinguish between the results for purified JEV purified from mouse brain treated with protamine sulfate treatment and JEV prepared from cell culture not treated with protamine sulfate. Liau *et al.* is silent regarding any difference in the neutralizing antibody titre of virus particles prepared from cell culture in comparison to virus particles prepared from mouse brain.

Combination of Huiying *et al.* and Liau *et al.*

6. Huiying *et al.* teaches inactivation of a vaccine, chemical purification by protamine sulfate precipitation to remove the cell DNA, and physical purification by zonal centrifugation to remove non-viral protein.

Liau *et al.* teaches physical purification of a JEV infected cell culture, which is then inactivated.

To prepare the vaccine of the present invention and obtain the , one would need to omit the physical purification step disclosed in Huiying *et al.* and still obtain a neutralizing antibody titer of the anti-serum obtained by immunization with the virus particles that is about twice to about 10 times the neutralizing antibody titer of the anti-serum obtained by immunization with inactivated virus particles prepared from virus cultured in mouse brain.

Those of skill in the art believed at the time that the step of chemical purification was necessary to remove the Vero cell DNA and would not be motivated to omit the step. Liau *et al.*, although omitting the chemical treatment step, shows a different order of steps. Liau *et al.* further is silent regarding any difference in the neutralizing antibody titre of virus particles prepared from cell culture in comparison to virus particles prepared from mouse brain. Thus one of skill in the art has no way of knowing if one would achieve the high neutralizing antibody titer found with the precise order and steps of the present invention. Therefore, there is no motivation to omit the chemical treatment step of protamine sulfate precipitation.

7. In summary, it is my professional opinion that the one of skill in the art would not be motivated to combine the teachings of Huiying *et al.* and Liau *et al.* according to the presently claimed invention.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the Unites States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

May/14/2004
Date

Asato Kojima
Asato KOJIMA, Ph.D.